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## The effect of grazing exclusion over time on structure, biodiversity, and regeneration of high nature value farmland ecosystems in Europe



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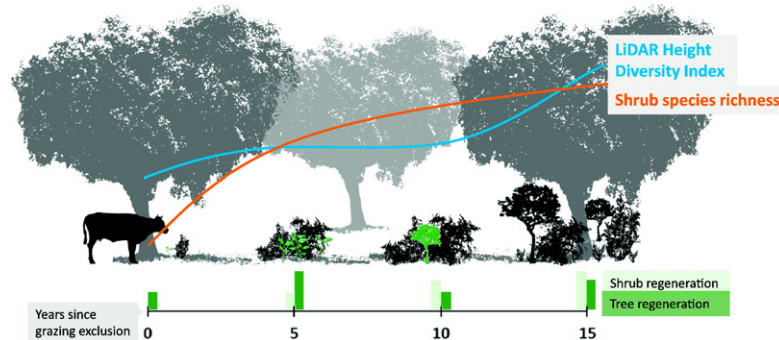
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### HIGHLIGHTS

- Grazing exclusion has direct impact of the *montado* forest structure.
- Structural and compositional diversity increase with post-exclusion time.
- 3-D spatial arrangement of structural elements represents forest dynamics.
- A structural diversity index is validated as a surrogate of ecosystem function.
- 5-Year exclusion enhances shrub diversity and regeneration and tree establishment.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Climate change and increasing socio-economic pressure is placing many ecosystems of high ecological and economic value at risk. This is particularly urgent in dryland ecosystems, such as the *montado*, a multifunctional savannah-like system heavily modeled by grazing. There is still an ongoing debate about the trade-offs between livestock grazing and the potential for ecosystem regeneration. While it is consensual that overgrazing hinders the development of the shrubs and trees in this system, the effects of undergrazing or grazing exclusion are unclear. This study provides the unique opportunity to study the impact of grazing on compositional and structural biodiversity by examining the ecological chronosequence in a long-term ecological research site, located in Portugal, where grazing exclusion was controlled for over 15 years. As the threat of intensification persists, even in areas where climate shifts are evident, there is a critical need to understand if and how the *montado* might recover by removing grazing pressure. We evaluate succession on structural and compositional diversity after grazing pressure is removed from the landscape at 5, 10, and 15 years post-cattle exclusion and contrast it with currently grazed plots. A LiDAR-derived structural diversity index (LHDI), a surrogate of ecosystem structure and function first developed for the pine-grassland woodland systems, is used to quantify the impact of grazing exclusion on structure and natural regeneration. The distribution of the vegetation, particularly those of the herbaceous and shrub strata ( $>10 \leq 150$  cm), presents statistically significant changes. The LHDI closely mimics the compositional biodiversity of the shrubs, with an increase

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in diversity with increased years without grazing. Under present climate conditions, both shrub regeneration and the establishment of tree saplings were strongly promoted by grazing exclusion, which has important management implications for the long-term sustainability of *montado* systems.

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## 1. Introduction

Climatic changes have increased environmental pressure on dryland ecosystems (Costa et al., 2011a), a particularly troubling fact for Mediterranean high biodiversity systems. By 1990, over 70% of the original extent of Mediterranean woodlands and scrublands has been converted to other land uses by longstanding human footprint (MEA, 2005). Although rural abandonment has allowed a gradual recovery of certain forested ecosystems, the evergreen oak forests of the Iberian Peninsula are consistently losing spatial coverage (Plieninger, 2006), consequently impacting their ability to provide ecosystem services and conserving biodiversity (Ferraz-de-Oliveira et al., 2016).

*Montados* (in Portugal) and *Dehesas* (in Spain) cover ca. 3.5 million ha (Pinto-Correia et al., 2011) and are multifunctional savannah type systems with highly variable evergreen canopy densities (*Quercus suber* or *Quercus ilex* spp. *rotundifolia*) and semi-natural grassland undercover (Sales-Baptista et al., 2016), currently dominated by grazing as a land-use (Bugalho et al., 2011a). The *Montado* is an agropastoral system that originated from natural forests following the removal of trees by human activities such as clearing, burning and grazing (Pulido et al., 2001). When the agricultural practices are removed or reduced, the succession leads to increases in woody plants (Castro and Freitas, 2009). Unlike other systems, the *montado* has been shaped by human activities for centuries (in fact close to a millennium or more in some areas) and is structurally kept as a savannah by human and not by natural factors. The succession of woody vegetation with decreasing land use intensity, including undergrazing, is well accepted for this particular ecosystem; common shrub control strategies such as shrub clearing and rotational ploughing are used to control woody succession (Pulido et al., 2001; Plieninger et al., 2003, 2004; Calvo et al., 2005), and even manmade fire (Godinho et al., 2016).

Managing the spatial patchiness, and multifunctionality of the traditional landscape (Tenhunen et al., 2009) of the *montado* is crucial to maintain the ecosystem services and associated socio-economic impact of the region (Concostrina-Zubiri et al., 2017). These ecosystems, critical for providing both high social value and biodiversity, are classified as High Nature Value (HNV) and their sustainable management is reliant on appropriate low intensity use (agricultural or animal stocking rates) and appropriate monitoring to ensure biodiversity levels (Beaufoy and Cooper, 2009). Even though the *montado's* high conservation value is protected by the European Union (Habitats Directive 92/43/EEC), these systems are facing significant threat from a shift from the traditional low intensity land use to either abandonment or intensification of land use (Sales-Baptista et al., 2016). Overgrazing is suspected to contribute to the long-term degradation of *montados*, reducing biodiversity and natural oak regeneration (Plieninger et al., 2010; Ribeiro et al., 2010; Bugalho et al., 2011a, 2011b; Concostrina-Zubiri et al., 2017). Land abandonment is also reported to have a strong effect on the vegetation composition with decreases in species diversity with decreasing land use intensity as a direct consequence of the replacement of short-lived herbaceous species with the dominant shrub *Cystus ladanifer* (Castro et al., 2010). As the land degradation of these systems accelerates, as evidenced by tree decline symptoms since the 1980s (Costa et al., 2011a) these economically vital ecosystems might cease to function (Doughill et al., 2010).

Grazing ecosystems evolved with and depend upon herbivory, heavy hoof action, nitrogen deposits, and decomposing carcasses of large migratory herbivorous. When introduced into ecosystems that did not evolve with frequent grazing, these forces can alter biological communities and ecosystem function. In human-controlled grazing systems, such as the *montado*, the detrimental or beneficial effects of grazing are largely determined by how and where grazing is used. The ecological impacts of grazing depend on the type of ecosystem, plant community, and conditions of a particular site. One of the ecological impacts of grazing is overgrazing, which is widely known to have an effect on composition and structure of plant communities and of biological soil crusts (Concostrina-Zubiri et al., 2017), by reducing biomass, soil nutrient enrichment and overall regeneration (Yan and Lu, 2015; Zhou et al., 2005).

Grazing exclusion is actively being used as a management strategy to prevent ecosystem degradation, promote high function, or even for restoration of degraded areas (Mata-González et al., 2007; Mofidi et al., 2013; Wei et al., 2012). For *montados*, both the overuse, in fertile areas, and abandonment of grazing, in less fertile and peripheral areas, pose a significant threat of ecosystem degradation (Pinto-Correia and Godinho, 2013; Bugalho et al., 2011b). Even though grazing exclusion is widely used for managing certain ecosystems, the impact of this land management strategy on plant structure, biomass, and biodiversity in the *montados* is still unclear. For some degraded grassland ecosystems, grazing exclusion appears to improve herbaceous cover, biomass and biodiversity including biological soil crusts (Mata-González et al., 2007; Modifi et al. 2013; Concostrina-Zubiri et al., 2017); other studies found no significant change in biodiversity with grazing exclusion (Yan and Lu, 2015) or even a decrease in species richness and biodiversity with exclusion (Bugalho et al., 2011b; Mayer et al., 2009; Shi et al., 2013). Many of these studies have focused on short-term exclusion (<6–8 years) and ecological processes that led to functional and biodiversity changes are longer term. Our study focuses on the progression from grazing to longer term grazing exclusion (from 0 to 15 years), focusing on the impact to the forest structure, biodiversity and regeneration.

Even though grazing is well accepted as a major ecological driver of the *montado* ecosystem, there is still a critical shortage of information on how to use grazing as a tool to promote biodiversity (Bugalho et al., 2011a; Azeda et al., 2014). Even more surprising is the lack of information on the regeneration of the tree stratum, particularly the cork oak, and the dynamics of its succession stages (Pausas et al., 2009) in the *montado* ecosystems. Most of the *montados* present an even-sized class distribution (Pausas et al., 2009) with few, if any, young trees. Lack of tree regeneration indicates that these ecosystems with high biodiversity potential are not currently being managed with sustainability in mind. Exclusion from grazing, particularly short-term (2–5 years) has been suggested as a land management technique in the *montado* ecosystem to allow tree regeneration (Bugalho et al., 2011a; Ramírez and Díaz, 2008), but this needs further testing in different situations for different components of the ecosystem.

There are other important consequences of removing grazing pressure in the *montado* ecosystem that must be managed as well. Removal of grazing pressure yields an increase in total above-ground biomass and net primary productivity, increasing the overall carbon storage of the system while making the system more likely to burn at higher intensity (Castro and Freitas, 2009). While cork oak is a pyrophyte, intensive wildfires can

damage the tree cork and are important variables influencing montado loss (Godinho et al., 2016). Typical management of the montado, using grazing or mechanical shrub clearing, lead to an ecosystem that has been traditionally low fire risk (Pinto-Correia and Mascarenhas, 1999).

To track the complexity of the *montado* system dynamics at the landscape scale, effective indicators must be developed to relay the changes in several strata simultaneously since these are affected by grazing at different rates and with variable response times. The pasture, shrub, and tree strata present different impacts from land management strategies, even though these are interrelated. The degree of tree and herbaceous cover in these *montados* is directly linked to ecosystem services, in terms of natural products (cork, acorn crops, animal feed, livestock, charcoal and firewood, cereals, tannins) (Joffe et al., 1999), and ecological functions (i.e. carbon and nitrogen storage, hydrological cycles, soil retention/nourishment, climate buffering, and biodiversity). Shrubs are critical for functional diversity, habitat for wildlife, and natural regeneration (Ferraz-de-Oliveira et al., 2016). Herbaceous cover is determinant for livestock grazing and to protect the soil from erosion (Nunes et al., 2017), and is critical for energy flow and nutrient cycling (Gilliam, 2007). The ability to manage these ecosystems to preserve and expand as HNV farming systems is closely related to maintaining high structural diversity using low intensity, low input management efforts (Azeda et al., 2014). With the rapid development of Light detection and ranging (LiDAR), quantifying the 3D structure of forest canopies is cost-effective, fast, and accurate (Van der Zande et al., 2008). As portable LiDAR units are developed at more accessible costs, a highly repeatable technique can provide the means to closely monitor the impact of management actions on all the strata simultaneously (Listopad et al., 2011). LiDAR data provides the ability to examine detailed vertical profiles of plots over time. As changes in land management (i.e. increases or decreases in grazing pressure, cropping, clearing, etc.) are applied to the system, these profiles provide the ability to closely track and potentially better understand track ecosystem dynamics and succession.

Many landowners, under incentives for sustainable management of *montados* (i.e. Forest Stewardship Council certification scheme), are looking for specific guidance on how to manage their land to promote biodiversity and ecosystem services. Adaptive management, guided by a close monitoring system that triggers an early warning system prior to reaching critical ecological shifts. Others have suggested the need to monitor grazing pressure continuously using wireless sensor networks (Sales-Baptista et al., 2016), focusing on alarming signs such as changes in functional plant types (Lavorel et al., 1999; Nunes et al., 2017) or increases in bare ground cover and animal trail densities (Walker and Heitschmidt, 1986). We suggest the use structural diversity indices, easily extracted from LiDAR datasets, as a proxy to compositional and functional biodiversity. This LiDAR-derived structural diversity index (LHDI) was developed for another highly-managed savannah-type ecosystem in the southeastern US (Listopad et al., 2015) as a surrogate for ecosystem biodiversity.

Our aim in this study was to assess the effects of grazing exclusion on forest structure, biodiversity, and recruitment using a chrono sequence of succession plots located in a *montado* Long-Term Socio-Ecological Research Site using LiDAR. Our focus was on responding to the following questions:

- 1) Does the structural composition of the cork-oak forest plots change significantly after 5, 9, and 15 years of grazing exclusion? If so, are the changes more important in the herbaceous, shrub, or tree strata?
- 2) Is there a promotion in forest structural biodiversity, measured with a LiDAR-derived Shannon-based index, with increases in grazing exclusion periods?
- 3) Specifically, for the shrub strata, is structural diversity index correlated with compositional diversity in the form of species richness, so structural LiDAR-derived indices could be used as an importance proxy for overall forest biodiversity?

- 4) Does grazing exclusion promote natural regeneration of shrubs and/or trees, and if so, how does this regeneration change with time post-exclusion?

The following hypotheses are being tested in this study:

- 1) Statistically significant structural changes take place with exclusion of grazing and increases in grazing exclusion interval increase the structural diversity of the montado
- 2) Compositional biodiversity increases at the shrub stratum with increases in grazing exclusion interval
- 3) Natural regeneration of shrubs and trees is promoted by excluding grazing
- 4) Compositional and structural diversity, as measured by the LiDAR structural diversity index, are correlated for the shrub stratum; this would allow the use of the index as a surrogate for compositional biodiversity

## 2. Material and methods

### 2.1. Study area and experimental design

The study area is a cork-oak *montado* site at the state-owned farm, Companhia das Lezírias, SA, a *Montado* Long-Term Socio-Ecological Research Site (LTSE), located 25 km north of Lisbon, Portugal (38° 52' 0.780 N 08° 51' 0.747 W) (Fig. 1). The climate in the study area is Mediterranean, with rainy cool winters and dry hot summers, and mean annual precipitation of 608 mm and mean annual temperature of 16 °C.

The LTSE sites, spread over a large spatial scale, are designated for long-term socio-ecological research, with the main objectives of monitoring and storing relevant data in ecology, establishing links among institutions and researchers and promote the exchange of data and knowledge. As for LTSE *montado* platform, the research objectives include increasing the understanding of local scale pressures and their mechanisms, providing tools to monitor large-scale drivers of change, and analyzing the socio-economic drivers in the ecosystem's long-term sustainability. The sites integrating the LTSE *montado* platform provide a unique set of human-shaped ecosystems, where both silvicultural and forestry are key components, representing a highly variable system in terms of climate-soil interaction, tree density, and land use patterns.

Since the XIX century, Companhia das Lezírias has been managing cork-oak woodlands for cork extraction, cattle raising, and other uses ranging from hunting to biodiversity conservation. No prescribed or wildfire occurs on this property for several decades, since it is a state-owned farm with high level of dedicated resources. Due to its interest

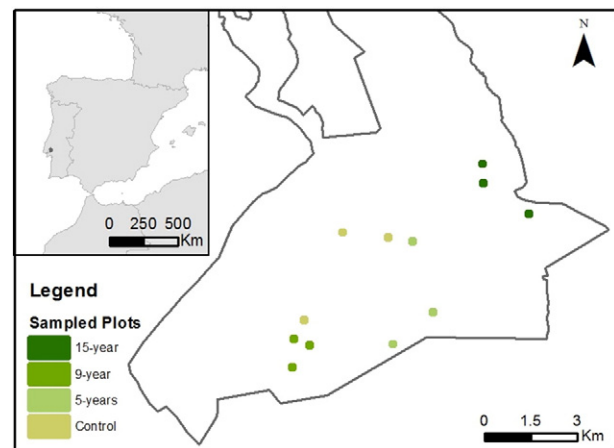


Fig. 1. Location Map of the plots within the study site, the Companhia das Lezírias, Santarém, Portugal. Four treatment types are represented with three replicates each: grazed or control plots, 5-year, 9-year, and 15-year exclusion plots.

in preserving cork oak woodlands ecosystems, the Companhia das Lezírias has been incorporated in the LTSE *Montado* platform as a strategic area of long-term monitoring, consistently testing best methodologies to use for sampling and selecting most appropriate indicators for long-term monitoring.

Twelve plots selected for this study were located within the site's *montado* ecosystem, with an average density of 85 trees/ha and canopy cover that typically ranges between 24 and 49% (Príncipe, unpublished data) (Fig. 1). In the past few years, some patches with dense shrubby understory in varying successional stages, totaling 1510 ha, have been fenced for cattle exclusion and have, subsequently, been managed for habitat protection and promotion of cork oak's natural regeneration. Grazing exclusion and avoidance of shrub clearance in the fenced landscape provide a unique opportunity to explore the mechanisms of *montado* succession and the resulting structural and functional changes over time.

The current experimental design is a randomized complete block design with four treatments and three replicates for each: control plots (where herbivory is not excluded) and 5, 10, and 15 years post-herbivory. Plot selection focused on the controlling multiple variables, such as soil type, inclination (topography), and edge effects from roads and intensive land uses. All plots were located >50 m from any roads and other land uses to limit exposure to any potential edge effects. Each plot was 1 ha in area (100 m × 100 m) and were surveyed for structural characteristics, shrub composition, and regeneration. Portable LiDAR derived variables include percentage cover and mean height of each treatment plot and by strata (herbaceous, shrub, and tree strata). In addition, LiDAR-derived data allowed the extraction of plot-based and strata-based LHD, a structural biodiversity index. Traditional field collected data included the following variables: shrub and tree species composition and cover, allowing the extraction of compositional richness and shrub/tree regeneration. More details on these two types of data acquisition methods are described in Sections 2.2 and 2.3.

## 2.2. Field composition and structural survey

The field data collection focused on structural and compositional variables of the understory woody vegetation, i.e. shrubs and young trees (up to 3 m tall). In each 1 ha plot, 4 transects with 50 m were distributed systematically in such a way that heterogeneous patches would be represented by the sampling effort, totaling 200 m per plot. All woody individuals intercepted by the transects were identified by species and the length of interception was registered (following the line-intersect transect method). Species richness was calculated as the total number of unique species recorded in the 4 transects per plot. Species cover was calculated by summing the length of all individuals of the same species per plot. Additionally, the height of all the intercepted individuals was measured using a tape-measure and recorded per unique individual.

## 2.3. LiDAR structural survey

Portable LiDAR data were collected in April–May 2013 for all 15 plots using a Riegl LD90-3100 HS eye-safe (laser safety class I) first-return type rangefinder operating at 890 nm and 1 kHz, connected to a lightweight Toughbook and placed in a lightweight backpack with home-built frame. The beam divergence of this profiling system is consistent with the manufacturer's specifications at 2.0 milliradian [35]. This is a very similar setup to the one used by Listopad et al. (2011), after being modified from the original Park et al. system (2004) to improve portability. The system was suspended in a backpack style frame that allowed adjustments to minimize tilting but the whole system was fixed and not gimballing. Even though the verticality of the laser was assessed prior to each plot data collection, tilting is a potential source of error, especially with the instrument mounted as a backpack. Due to the interest in obtaining shrub and herbaceous layer data, the

backpack system was further modified to allow the system to swivel and collect data facing upwards (tall shrub and tree data) and downwards (small shrub and herbaceous data). This Riegl rangefinder averages a minimum of five ranges together to give one measurement, and presents “sky hits” (open canopy) as an error, allowing for easy accounting of open canopy returns. Another modification to this portable LiDAR system since 2011 has been the incorporation of x and y positional information along with the measurement information. A Trimble GeoXT (submeter) unit was used in conjunction with the portable unit for LiDAR data collection, with the measurement data being tagged every 10 s with new positional information. This allowed most data to have x, y, and z data, with exception of technical difficulties and heavy canopy areas, where GPS signal was difficult to obtain or highly inaccurate.

To represent the structural distribution of the vegetation of the 100 m<sup>2</sup> quadrats as accurately as possible, 10 transects, placed strategically every 10 m, were collected per plot for both upwards and downwards data collection. This 10 m distance corresponds to the maximum spacing between returns. Within the acquired transects, point spacing is minuscule in this type of continuously profiling system.

The data are recorded in an ASCII text file format using a serial data connection, and appropriately labeled for each plot. Even with the ability of obtaining positional location, we attempted to control the walking speed to provide similar number of LiDAR returned per transect to accurately represent the structural composition of each quadrat. This portable LiDAR system was redesigned from the one used by Parker et al. (2004) [35] to include an on/off switch. This allows the data collection to be paused temporarily and resumed when there are difficult field conditions, such as heavy understory cover and impassible ditches, reducing the bias of collecting high data density in difficult to pass areas and also reducing deviations to the predetermined transects. Even with the use of the switch, maintaining constant speed and/or trajectory within a predetermined transect are difficult, and applications of this type of data should not depend on positional accuracy. With the addition of xy locations to the height (z) data, density of the data collection per plot can be better controlled, and bias of speed of the data collection is less of a concern. Ability to view the data points as x,y,z in a plot pSmall movements in direction or position of the collector could change the exact target of the laser. However, for this particular study, since xyz are not used for individually collected data points and analysis is based on the aggregation of all points per plot, these potential small changes in target should not have an impact on plot level studies.

## 2.4. Data analyses

Data was processed independently for the traditional field data collection of shrub and recruitment data and portable LiDAR data. Subsection 2.4.1. described the traditional field collected data analyses processes. The LiDAR data was processed and analyzed using statistical analyses according to the methods described in Section 2.4.2.

### 2.4.1. Traditional field data analyses

Total number of shrub species found per plot were calculated to derive the shrub richness for all 12 plots.

Tree regeneration was assessed by computing the cover and height of saplings and juveniles in each treatment. For shrub regeneration, we firstly computed, for each species, the height of the 90th percentile, assumed this value to be the typical height of an adult individual, and considered individuals below 20% of this height to be shrub juveniles. Shrub regeneration was then computed by summing the cover of all shrub juveniles.

Differences in regeneration between grazed plots and the different periods of grazing excluded plots was assessed by Kruskal-Wallis ANOVA using an  $\alpha = 0.05$ . If significant differences were encountered, such as for the heights of young trees, a post-hoc Dunnett test ( $\alpha = 0.05$ ) was implemented to understand pairwise differences.

### 2.4.2. Portable LiDAR data analyses

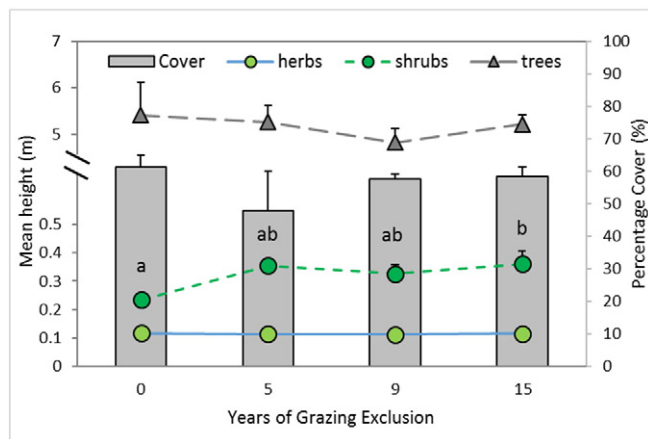
The portable LiDAR data collected in ASCII text file formats were merged by plot into database tables. Pre-processing of these data including assigning open/closed canopy indicators for all returns and adding 1.3 m (the height above ground of the portable LiDAR data collector) to all canopy return heights. Herbaceous data collection files were processed separately and distance to target was subtracted from the height of the LiDAR system (once swiveled towards the ground) measured at each plot. Since the data collected are very simple (distance to target), only spreadsheet and database software were used. Individual transect data were collected in separate text files, but aggregated per plot during analyses. Since  $z$  is provided in distance to target (i.e., vegetation), planar differences among transects should be inherently accounted for. Upwards and downwards data were analyzed separately for certain metrics (canopy cover only used the above 1.3 m dataset), but mostly combined to obtain a complete vegetation profile from the three vegetation strata. Herbaceous stratum was defined as all the vegetation collected between 0 and 15 cm in height, shrub and small tree stratum was composed of vegetation >15 and ≤150 cm, while tree stratum was defined of all the vegetation >150 cm.

The following metrics were calculated for the portable LiDAR data: canopy cover, vegetation height (maximum, minimum, mean, and standard deviation) for the entire profile and each stratum, and a LiDAR-derived diversity index (LHDI based on the standard Shannon-Height Diversity Index Formula (Listopad et al., 2011 and Listopad et al., 2015)). The canopy cover for the portable LiDAR, included all captured canopy returns (>1.3 m) divided by the total returns (open and canopy returns). The structural indices for the overall canopy profile and the tree stratum were calculated using the proportion of returns within every 0.5 m interval, while the proportion of returns for the herbaceous diversity indices used 1 cm intervals and the shrub indices used 5 cm intervals.

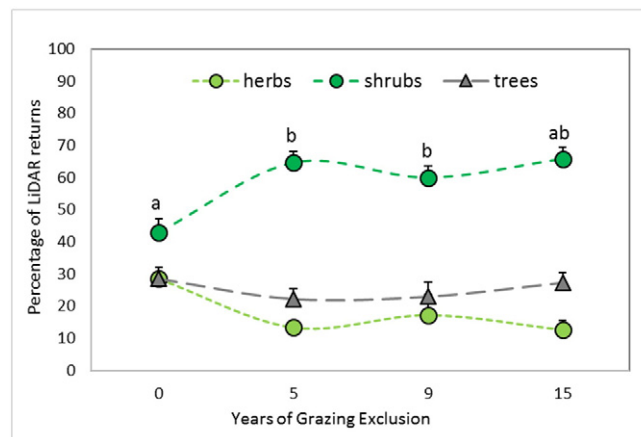
Canopy cover, mean height and LHDI by strata for the treatment types (control, 5-, 9-, and 15-year grazing exclusion) were tested for significant differences using one-way ANOVAs ( $\alpha = 0.05$ ). If differences were encountered, the Tukey post-hoc test at  $\alpha = 0.05$  was used for pairwise comparisons. Cumulative distribution of LiDAR returns by strata was averaged for all the replicated per treatment type and plotted for comparison of structural shifts along the chrono sequence. Shrub diversity is correlated with the shrub LHDI and Spearman's correlation coefficient for linear regression is reported.

## 3. Results

Canopy cover, derived from the LiDAR-collected dataset, varied between 40.7 and 79.8% for all 12 plots. No statistically significant



**Fig. 2.** Canopy cover (%), and mean height for the herbaceous, shrub, and tree strata by treatment type derived from the LiDAR profile data. Significant differences in mean shrub height using a One-Way ANOVA indicated using Tukey's post hoc test ( $p = 0.043$ ,  $\alpha = 0.05$ ).



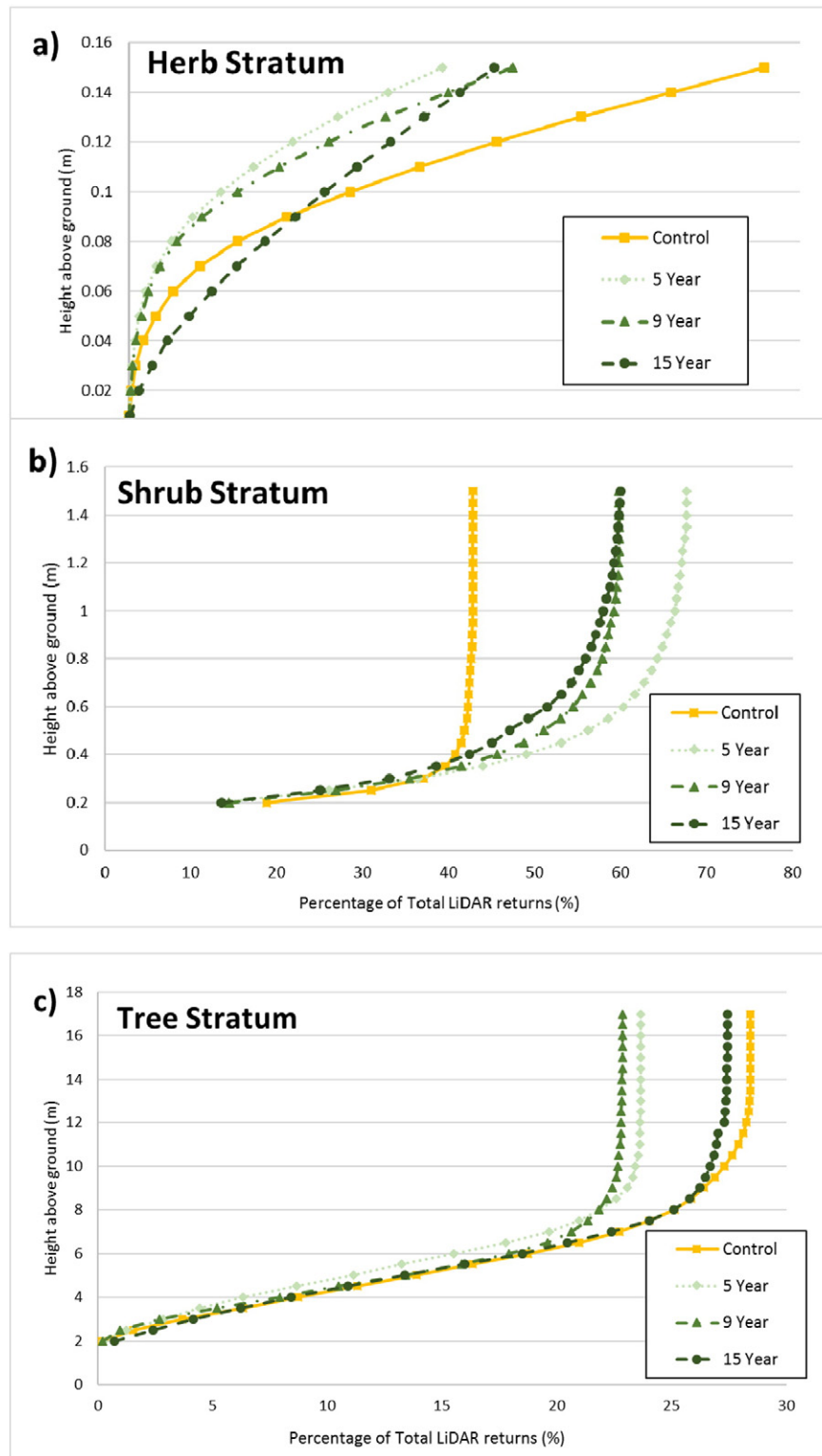
**Fig. 3.** Distribution of the LiDAR returns for the herbaceous, shrub, and tree strata by treatment type. Significant differences in the shrub mean percentage using a One-Way ANOVA indicated using Tukey's post hoc test ( $p = 0.043$ ,  $\alpha = 0.05$ ).

difference was found in the canopy cover among the different treatments ( $p = 0.79$ ,  $\alpha = 0.05$ ); visually only the 5-year post exclusion treatment had medians below 50% canopy cover (47.9%), while the remaining treatments, including the grazed plots, has medians above 58% (Fig. 2). Differences in the mean height for the herbaceous and tree strata were also non-significant among treatments (Fig. 2), consistent with the small variations observed in the percentage of the LiDAR returns for each of these strata (Fig. 3).

Variations in the shrub layer, measured both as the mean shrub height and the percent distribution of the total vegetation within the 15–150 cm height class, are statistically significant among the treatment years ( $p = 0.043$  and  $p = 0.013$  @  $\alpha = 0.05$ , respectively). With the removal of grazing pressure, the shrub vegetation quickly recovers, and at 5-years post grazing exclusion an increase of 16% of the total vegetation distribution is within this height class (Fig. 3). An increase in the shrub layer, particularly in the mid-range (30–60 cm in height), causes the mean shrub height to increase from grazing to post-grazing years from 21 cm to 34 cm (Fig. 2).

Post-hoc analyses indicated that the grazed mean shrub height is significantly smaller (23 cm) than the 15-year post exclusion shrub height (36 cm). The intermediate treatments, 5- and 9-year post grazing exclusion present intermediate shrub mean heights (35 and 33 cm, respectively) with enough within-treatment variability not to be significantly different from either the grazed or 15-year post exclusion plots.

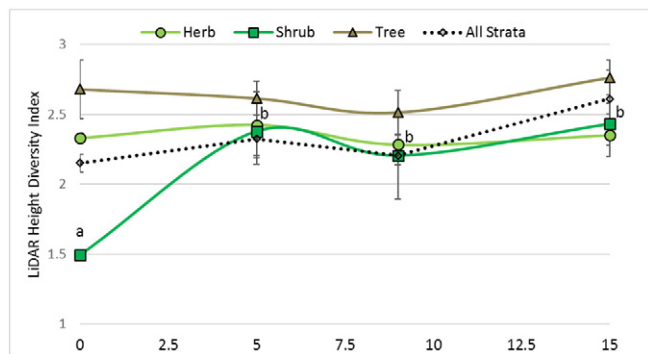
The cumulative distributions of the LiDAR returns present the strongest shifts in patterns post grazing exclusion in the shrub stratum (Fig. 4b), but differences, though less visible, are present in the herbaceous and tree strata (Fig. 4a and c, respectively). The herbaceous cumulative distribution indicates that the 10% of the total plot vegetation is under 10 cm in height while grazing is present, 13 cm 5-years post grazing, and 12 and 11 cm 9- and 15-years post grazing (Fig. 4a). The shrub cumulative distribution indicates that most of the shrubs are under 40 cm and represent ca. 40% of the total plot vegetation in the control plots; in contrast, 5-year plots have almost 65% of the plot vegetation distributed up to 80 cm in height, 9- and 15-year plots have 55% and 57% of the vegetation distributed up to 80 cm heights (Fig. 4b). Differences in the cumulative distribution of the large shrub and tree stratum mostly represent maximum tree height, with taller vegetation found in the control and 15-year post-exclusion plots (ca. 12 m) and shorter (ca. 10 m) in the 5- and 9-year post exclusion plots. A larger percentage of the total vegetation is present in the control sites (28%) since the shrub component is reduced from the grazing pressure. As grazing exclusion is extended, however, the canopy component presents many similarities to the control plots, since recruitment of the trees hasn't reached heights much beyond the 150 cm in height.



**Fig. 4.** Mean cumulative distribution of LiDAR returns (as a percentage of the total returns) for the control, 5-year, 9-year, and 15-year treatment type by stratum: herbaceous 0–15 cm (a), shrub >15 ≤ 150 cm (b), and tree >150 cm (c).

Forest structural diversity is also affected by grazing, with overall increases in diversity with time post grazing exclusion (Fig. 5). Examining the changes in structural component by stratum, the most significant differences are the ones, once again, for shrub component, with increases in the LHD from 1.49 for the grazed plots to 2.43 for the 15-year post exclusion plots ( $p = 0.002$ ;  $\alpha = 0.05$ ). Shrub stratum was the most dynamic thus we will focus further

biodiversity analysis on this woodland component. The shift in the structural diversity of the shrub component takes place soon after grazing is removed, with significantly lower diversity values while grazing is taking place than in ungrazed plots. Increasing the time lapse much beyond 5 years does not appear to increase the structural diversity values of the shrub significantly. Herb structural diversity changes are similar for all treatments (between 2.24 and 2.31),



**Fig. 5.** LiDAR Height Diversity Index (LHDI) for the herb, shrub, and tree strata, as well as the overall profile by treatment type. Significant differences in the shrub LHDI using a One-Way ANOVA indicated using Tukey's post hoc test ( $p = 0.002$ ,  $\alpha = 0.05$ ).

with very small variation among and between plots. Tree structural diversity is much more variable and doesn't present a significant trend, with only slightly higher diversity values for the 15-year treatment (2.76) versus all other treatments (2.51–2.68).

Overall increases in structural diversity of the shrub stratum correlate significantly with both shrub species richness ( $r = 0.727$ ,  $p = 0.007$ , Fig. 6) and the Shannon's index for shrub species ( $r = 0.774$ ,  $p = 0.003$ ). Species richness numbers were as low as 1–4 in the grazed plots and rapidly increased to 9–11 in 5 years of exclusion and up to 15–17 species after 15 years post-exclusion. A similar linear increase in structural biodiversity was found for the shrub component with an LHDI of 1.49 for grazed plots, rapidly increasing to 2.38 5 years post-exclusion and up to 2.43 after 15 years post-exclusion.

Shrub regeneration appears to be completely inhibited by grazing with immediate increases as soon as the pressure is removed from the cork-oak system (Fig. 7). The cover of young shrubs increases with absence of grazing pressure, from 0% recruitment in the control plots, 0.48% in the 5-year plots, 0.88% in 9-year plot and reaching a peak recruitment of 1.17% in the 15-year post exclusion plots. Tree regeneration, on the other hand, is present for all treatment plots. Cover of young trees shows a non-significant increasing trend, with 0.78% in the control plots, 2.30% in the 5-year, 4.21% in the 9-year and 5.49% in the 15-years exclusion plots. However, the height of those young trees is clearly different among treatments. Plots excluded from grazing for 9 and 15 years have significantly higher trees than grazed and 5-years excluded plots.

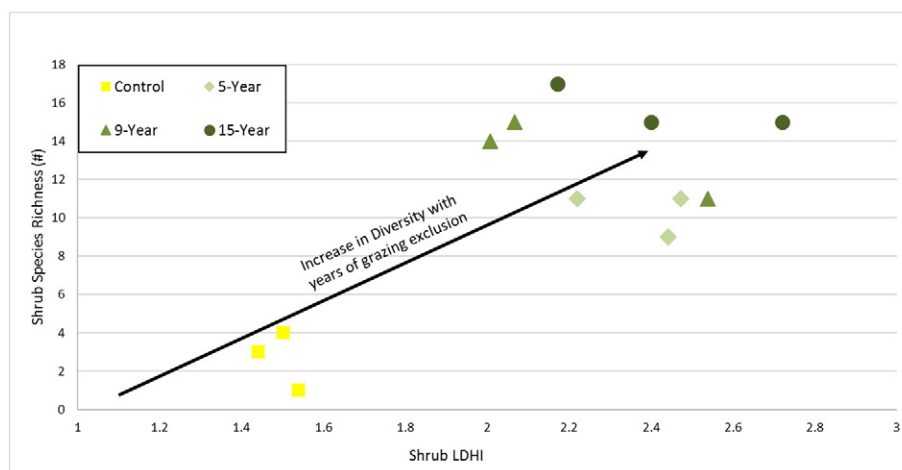
## 4. Discussion

### 4.1. Structural impact of grazing exclusion

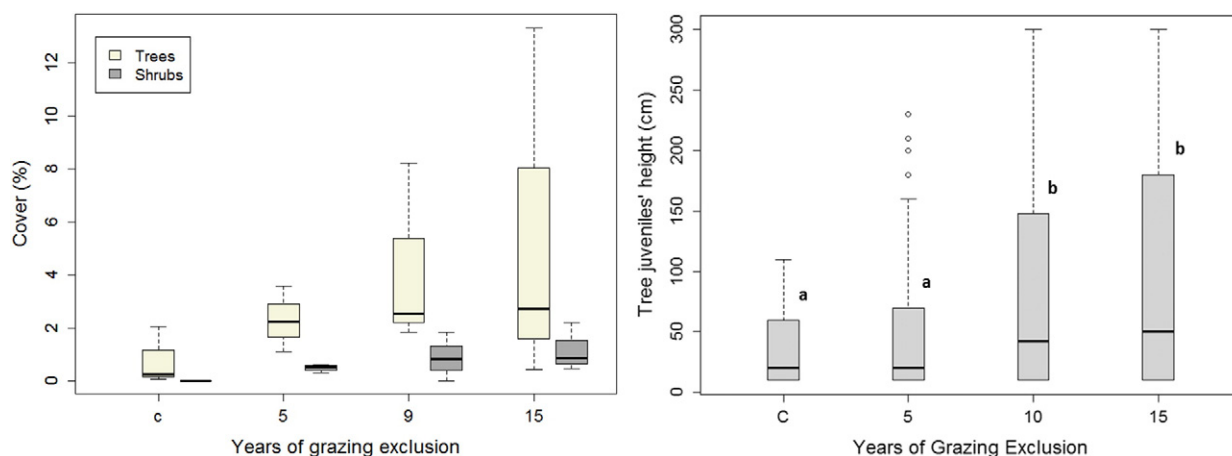
Grazing exclusion has no obvious impact on the mean canopy cover of the plots, as these were highly variable within the treatment types, particularly for the 5-years post exclusion one, where values ranged between 41% and 80%. High habitat heterogeneity, including changing composition and density of tree cover, is a typical characteristic of the *montado* (Ferraz-de-Oliveira et al., 2016). The plot with highest canopy cover (the 5-year plot with 80% canopy cover) has the greatest tall shrub component of all the plots, just above the 150 cm in height. The use of ground-based LIDAR and definition of returns above 150 cm in height as “canopy” returns might have biased the canopy cover values of these plots in comparison to traditional forest structure techniques.

The most dramatic shifts in the vegetation distributions take place in the first five years after the grazing pressure is removed, with a decrease in total herb-based vegetation from 9% to 4% and increase in shrub vegetation from 60% to 79% (Fig. 3). As the chrono sequence progresses, from 5 years to 15 years post-grazing, the shrub component starts to gradually decline, from the peak of 78% for the 5-year plots to 70% of the total plot vegetation for the 15-year plots. This is accompanied by an increase in the large shrub and tree component (> 150 cm) from 18% at 5-years to 25% at 15-years post exclusion.

The shrub component, a critical element for functional diversity and oak tree regeneration (Ferraz-de-Oliveira et al., 2016), clearly responded to grazing exclusion: grazed plots presented significantly lower percentage of shrub LIDAR returns (43%) than the 5-year and 9-year post exclusion plots (65 and 60%, respectively). The strongest effects were observed during the initial stages of grazing exclusion, consistent with other studied examining grazing abandonment in the *montado* (Castro et al., 2010) and other Mediterranean ecosystems (Peco et al., 2005). After 15-years, the shrub component reaches 66% of the total LiDAR returns, but there is significant variability in the structural composition within treatment plots. Unlike other savannah-like systems in the world, where overgrazing typically increases shrub encroachment (e.g. Van Auken, 2000; Bowman, 2002; Hughes et al., 2006), studies from Mediterranean systems such as the *montado* (Karakosta and Papanastasis, 2007; Zarovalli et al., 2007) have reported consistent increases in woody vegetation with decreases in land use intensity. Land use changes in the *montado* are mostly associated with secondary successional vegetation changes which mimic the return to the original natural forests prior to the removal of trees by human activities such as clearing, burning and grazing (Pulido et al., 2001).



**Fig. 6.** Significant Pearson correlation ( $r = 0.727$ ,  $p = 0.007$ ) between structural (LHDI) and compositional diversity (richness) for the shrub layer using all 12 study plots. Plots are symbolized by treatment type.



**Fig. 7.** Regeneration of shrubs and trees by treatment. A: Cover of shrubs and young trees, no significant differences among treatments, Kruskal-Wallis ANOVA  $p = 0.11$  and  $p = 0.27$ , respectively,  $\alpha = 0.05$ ; B: Height of young trees, significant differences among treatments tested with Kruskal-Wallis ANOVA,  $p = 0.003$ ,  $\alpha = 0.05$ , indicated using post hoc Dunnett test.

The rapid increase in the shrub component after grazing is excluded from the *montado* system and then subsequent stabilization after 15 years has significant management implications: removal of grazing, even in the short-term (5 years), can have an important impact of the forest structure, providing increase in above-ground biomass, functional support as wildlife habitat and, most likely, the tree regeneration. The shift in life form dominances also result in the replacement of Mediterranean annual grasslands communities with shrub communities, which has further implications in carbon and nutrient cycling (Castro et al., 2010).

#### 4.2. Biodiversity and system dynamics

The cumulative distribution curves, derived from LiDAR profiles, summarize the dynamics of the entire *montado* forest structure in one snapshot, an important holistic approach to provide appropriate indicators to managing these forests for biodiversity and sustainability. Understanding the dynamics of the vegetation components through time, as a result from several types of disturbances, is possible with this more accessible remote sensing technique. In other savannah-type systems, disturbance might be fire, and prescribed fire is often guided by feedback mechanisms, including understanding of forest dynamics and structure (Listopad et al., 2011; Listopad et al., 2015). For *montado* ecosystems, grazing exclusion has been inadequately explored as a management tool (Bugalho et al., 2011a), mostly due to gaps in knowledge of its impact on forest dynamics, structure and biodiversity. The use of LiDAR techniques, such as the ones in this study, might provide a cost-effective and timely alternative to filling in these critical gaps in knowledge.

The cumulative distribution curves indicate that the dynamics of the different structural components of *montado* are very different. The herb and shrub components, critical for biodiversity, present a rapid response to the removal of the disturbance (i.e. grazing) at or below 5 years, with stabilization after 10 years post-disturbance. Other studies concluded similarly that the strongest effects occur in the initial stages after grazing pressure is removed, and these are most visible in the herbaceous and shrub components (Westoby et al., 1989; Castro et al., 2010). For the tree component, critical for regeneration, the response is slower, and a change in dynamics only becomes apparent 15 years after post-disturbance. This time gap post-exclusion matches with recommendations from others (Bugalho et al., 2011a) of using 15–20 year cycles for tree regeneration and/or as sources of plant and animal biodiversity (Benayas et al., 2008). Extending our sampling (or returning to our plots) after 20 or 25 years after grazing is removed would provide invaluable information on the dynamics of the forest structure in relationship to this ecological driving factor. Other important ecological drivers,

such as interspecific competition for light and other resources, would also become more apparent in mid to late succession treatments.

Removing grazing pressure in these cork-oak systems yields an increase in both structural and compositional biodiversity of the shrubs, which is critical for recruitment of the economically important *Quercus suber*. This clear increase in plant species richness of the shrub component after removal of grazing from the *montado* system is in contrast to other studies (Bugalho et al., 2011a) that show no significant difference between grazed and ungrazed (<2 years post-grazing) plots. It is important to point out, however, that our study focused on the species richness and compositional diversity of the shrub component only, since this stratum is critical to maintain the High Nature Farming System (Azeda et al., 2014) and functional diversity. Even though the herbaceous component of the *montado* is critical for the overall system's diversity, its diversity is a direct reflection of livestock preferences and, in many cases, seeding as a management strategy to increase biodiversity.

Most studies that address the impact of grazing on biodiversity (Yan and Lu, 2015; Bugalho et al., 2011a) focus on grazed versus ungrazed plots and not on the dynamics of these changes on a temporal scale. The few studies that focus on removing grazing pressure on the *montado* ecosystem look at abandonment - of both cropping and grazing-, not purposefully exclusion of grazing as an ecosystem management technique. Castro et al. (2010) found that in intermediate succession plots after abandonment of grazing (10–15 years), species richness per plot, which included all three forest components, was significantly lower than the richness encountered in the currently grazed plots. Advanced succession stage plots (20–30 years post-grazing), presented even lower species richness values. Most of the decreases in species richness were driven by the replacement of the highly diverse grassland community maintained by low intensity grazing, with a monoculture of *Cistus ladanifer*. In contrast, our study shows that grazing promotes monoculture and dominance of *Cistus*, while its absence reduced the presence of this shrub species. Our study provides evidence that even in areas with long history of human management, such as the Companhia das Lezírias, the expected pathway of natural succession through gradual colonization of shrubs with oak regeneration (Correia, 2002) can take place. The concept of persistent alternative stages of succession (Westoby et al., 1989; Scheffer et al., 2001), such as the arrested development observed in Castro et al.'s study, needs additional research.

The apparently linear increases in both structural and compositional diversity of the shrub components after grazing pressure is removed are also potential indicators of increases in forest functions, such as carbon storage and nitrogen retention, highlighting the importance of selectively removing grazing pressure as a restoration technique (Listopad

et al., 2015). As in 2015 study, the structural diversity index follows other indicators of diversity such as species richness, showing that these indices might also be used as surrogates of species richness. Beyond monitoring grazing pressure to avoid land use intensification, considered the “true threat” to the *montado* (Pinto-Correia and Mascarenhas, 1999), a better early warning system might be monitoring this surrogate of forest plant biodiversity. Defining “overgrazing” by using a particular stocking rate number or totals grazing days might not provide sufficient data for successful adaptive management due to three reasons. First, defining what an appropriate level of grazing is site specific and dependent on soil conditions, microclimate, historic management, and other concurrent land management strategies. Secondly, we anticipate that these same appropriate targets will have to be adapted to climate change impacts in the region. Thirdly, a holistic indicator to guide adaptive management requires the spatial heterogeneity of the *montado* ecosystem to be accounted for. A combination of grazing pressure, type of grazing (sheep, cattle, etc.), timing (frequency and duration), and other applied management approaches (tilling, cropping, rotational system) have implications in the biodiversity and the type of succession that could be expected in the *montado*. It is likely that maintained small patches of grazed plots with early succession grazing-excluded habitats might promote highest plant and invertebrate diversity (Bugalho et al., 2011a).

#### 4.3. Potential implications for the tree regeneration of the *montado*

Tree regeneration is subject to a variety of factors: seed production, susceptibility to drought stress (Arosa et al., 2015), highly variable dispersal ranges (from a few meters to few kms, Pons and Pausas, 2007), highly variable predation by insects (Branco et al., 2002, Pérez-Ramos et al., 2007), and predation by large vertebrates. The sustainability of the *montado* is dependent on the cork oak recruitment; economically, cork income provides the most significant contribution, well ahead of cropping or livestock, in a cork *montado* (Pinto-Correia and Mascarenhas, 1999). The optimal conditions for cork oak recruitment and tree health are complex and often appear to be conflicting. Simões et al. (2015) concluded that cork oak recruitment was promoted by medium (40–60%) density shrub cover, and both sparse coverage and heavy shrub encroachment would affect oak regeneration. Pinto-Correia and Mascarenhas (1999) described the high livestock charge as a limiting factor in oak regeneration, while low frequency of shrub clearing affects the tree health of adult trees. Others (Bugalho et al., 2011a) suggest the importance of spatial heterogeneity, such as incorporating ungrazed patches of different rotations to promote tree recruitment.

Tree regeneration, measured as percentage cover, doesn't appear to be significantly different among grazed and non-grazed plots, which is surprising, but likely due to the high variability of the plots. Tree regeneration occurs continuously regardless of the period of grazing exclusion, because very short individuals are frequent along the chronosequence. Regeneration percentages are highly variable within treatment plots, so there are other variables beyond grazing presence or absence factoring in recruitment success. One of these key variables might be interspecific competition in the plots with longer exclusion periods (9+ years): as the shrubby component increases light becomes a limiting factor, likely hindering recruitment. It is also important to note that tree regeneration numbers include both pine and cork oak trees (observed data), which were likely captured at different stages of succession. Grazing appears to have a more significant impact on the shrub versus tree regeneration, although saplings establishment may be affected by grazing. The lack of shrub regeneration in grazed plots is clearly linked to the overall low shrub species and structural diversity of the same plots.

In order for the *montado* ecosystem to be sustainable, trees have to not only recruit, but grow enough in height to be protected from grazing reintroduction. From our data, the tree height of juvenile only

significantly increases after 9 years of grazing exclusion. For management purposes, excluding patches of the *montado* from grazing for only five years might not allow an increase of established trees, which is of critical interest for land owners.

Future threats of the *montado* ecosystem are directly linked to a continuous intensification of grazing, as the economic benefits of cork oak exploration decrease with lack of natural regeneration. These effects might be even further exponentiated with the recent climatic shifts in southern Portugal, where large portions of the landscape are *montados*. The last decades have characterized the regional climate as much drier than normal with a high incidence of wildfires (Costa et al., 2011b). From 2000 to 2010, climatic shifts have yielded rapid changes in water availability in southern Portugal, with the drought indices indicating a shift from sub-humid to semi-arid conditions. As the process of land degradation accelerates with both land use intensification and climatic shifts, adaptive management is key for the sustainability of this high value farming system. At the center of a proactive approach that encompasses the human-climatic interactions, there is a growing need for a reliable, repeatable, and cost-effective tool. As remote sensing techniques become more ubiquitous and cost-effective, structural information can allow proxy measures of functional biodiversity guide short and long-term management decisions.

## 5. Conclusions

Removing grazing, currently the most important disturbance factor in the *montado* ecosystem, has direct impact on this system's structural composition. This shift is only significant for the shrub stratum and particularly critical within the initial 5 years after grazing exclusion. The shrub mean height and percentage of LiDAR returns from the shrub component increase significantly after grazing is removed from the landscape. Extending the post-exclusion period to 10 and 15 years, only provides minor compounding changes to the shrubby component.

Shrub structural diversity, as measured using the LHD1, and shrub richness is also promoted with exclusion of grazing in the *montado*. The structural diversity increases the most in the first five years post-exclusion, while the compositional diversity presents an almost linear increase in total richness with greater exclusion periods. The relationship between structural and compositional diversity is significant, allowing the structural diversity index to be used as a proxy for shrub biodiversity. This proxy, particularly when focused on the shrub component might be a sensitive and cost-effective indicator to use when managing the *montado* ecosystem for biodiversity.

The sustainability of the *montado* is dependent on successful tree regeneration. Tree regeneration was present in both the grazed as well as in the ungrazed plots. However, the successful establishment of tree saplings, critical for the sustainability of the *montado* system was only noticeable after 9 years of exclusion. While 5 years post-exclusion promotes increases in the shrubby component and shrub diversity, longer exclusion periods (between 5 and 9 years) might be necessary to allow the tree saplings to gain enough height to be protected from future grazing reintroduction.

There are still critical gaps in knowledge that need to be studied to prevent ecosystem degradation. We propose that even longer-term (20+ years) dynamics of the forest components, such as changes in the structural and compositional biodiversity needs to be studied. In addition, vertebrate and invertebrate biodiversity assessments among the different grazed and ungrazed plots would provide valuable information for management with the goal of increase overall biodiversity. The recruitment and establishment of the cork oak trees needs to be tracked from pre-exclusion to 20+ years post-grazing exclusion. Studies with reintroduction of grazing after 5, 10, and even 15 years of grazing exclusion will provide guidelines for the best exclusion interval for restoring the *montado* ecosystem. It is only with science driven management decisions that these high value forests at high risk of degradation can be sustained in the long-term.

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